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**14. ABSTRACT**
Prostate cancer (PC) is the second leading cause of male U.S. cancer deaths, with African-Americans having the highest rate of PC mortality worldwide, as well as more abnormal results from screening tests that correlate with current or eventual PC. A 3-year prospective clinic-based study is studying the performance of current (PSA and DRE) vs. (% free PSA) clinical biomarkers of PC risk in 400 African-American men 50 to 70 years of age who undergo PC screening in Oakland, CA (East Bay San Francisco area), as well as possible association of PC screening results for these men with their dietary exposures to the cancer-causing heterocyclic amine, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) that forms when meat is cooked. This study expands an ongoing NIH-funded study (by the same research team) to add a new %-free-PSA test, results of which will be compared with PSA/ DRE results and PhIP exposures estimated by dietary interviews. For 392 men studied under the NIH protocol, an odds ratio (95% CL) of 32 (3.2, 720) for highly elevated PSA (≥20 ng/mL) was observed in the highest 15% vs. the lower 50% of estimated daily PhIP intakes. Approximately 100 additional men have completed participation in the expanded NIH/DOD-supported study. This study will help define the potential value of improved screening and dietary/behavioral intervention to reduce PC risk, namely, prevention of PhIP intake by avoiding overcooked meats.

**15. SUBJECT TERMS**
African-American men, cooked-meat carcinogens, epidemiology, PhIP, prostate cancer, screening

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## Introduction

PhIP is a Dietary Carcinogen that May Pose Heightened Risk to African-American Men

African American (AA) men, who compared to Caucasians die nearly twice as much from prostate cancer (PC), also take in about twice as much of the predominant U.S. dietary heterocyclic amine, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) (Bogen and Keating, 2001), which occurs primarily in well-cooked chicken and beef. Heterocyclic amines (HAs) are potent mutagens formed in meats, chicken and fish as it is cooked to higher-doneness levels by heat-intensive cooking methods (Thompson *et al*., 1987; Keating et al., 1999, 2000). HAs also cause cancer at a variety of sites in multiple bioassay animal species/strains/sexes, as well as at multiple sites within many of species/strains/sexes tested (Bogen, 1994). A predominant HA found in cooked and particularly in well-done chicken and beef is 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) (Felton et al., 1984, 1986; Felton and Knize, 1990a-b; Sinha et al., 1995). Dietary exposure to PhIP has been shown to induce colon, intestinal and mammary adenocarcinomas in rats (Ohgaki et al., 1986; Ochiai et al., 1991; Ito et al., 1991; Ito et al., 1997; Ghoshal et al., 1994), as well as prostate cancer in rats (Shirai et al., 1997,1999). HAs are metabolically activated by P450 and N-acetyltransferase enzymes to activated forms that bind covalently to (among other targets) DNA in tissues (including in prostate) where HAs induce cancer in rats (Thorgeirsson et al., 1983; Thorgeirsson, 1984; Rosenkranz and Mermeistein, 1985; Sato et al., 1986; Kato and Yamazoe, 1987; Snyderwine and Battula, 1989; McManus et al., 1990; Turesky et al., 1991; Davis et al., 1993; Kaderlik et al., 1994a-b; Takahashi et al., 1998; Schut and Snyderwine, 1999; Gooderham et al., 2002). In male *lacI* trangenic rats, a diet containing 200 ppm PhIP was shown to be highly mutagenic in prostate tissue (Stuart *et al*., 2000). In cultured human prostate tissue and primary prostate cells, PhIP is metabolically activated to mutagenic forms that covalently bind to and damage DNA (Williams et al., 2000; Lawson and Kolar, 2002; Martin et al., 2002; Di Paolo et al., 2005).

PC and African-American men

In the U.S., prostate cancer (PC) is a leading cause of cancer death among men, with African Americans having the highest age-specific prostate cancer rate in the world, and a >2-fold higher rate of mortality for PC than white men in the U.S.(Miller *et al*., 1996; Robbins *et al*., 1998; Hsing *et al*., 2000). Although family (particularly father/brother) history of PC is clearly linked to substantially elevated PC risk (Schuurman *et al*., 1999; Hemminki and Czene, 2002; Zeegers et al., 2003; Hemminki and Chen, 2005) and a human-PC-specific chromosome translocation has been identified (Tomlins et al., 2005), there is (as for most other cancers) no evidence for a predominant heritable factor for PC. Racial-ethnic differences in levels of testosterone-related hormones and related genetic controls on hormone-induced prostate growth have been hypothesized to explain or contribute to corresponding racial-ethnic differences in PC risk, but their role remains unclear (Ross *et al*., 1998; Pettaway, 1999; Hsing, 2001). Even if hormonally mediated background processes do affect PC risk, dietary exposures to genotoxic HA carcinogens may act independently or interact synergistically with such hormonal processes to further modify PC risk.

HA, Meat, Other Dietary Factors, and PC Risk

Substantial geographical variations in PC incidence indicate the importance of one or more dietary or other environmental factors (Minami et al., 1993; Mettlin, 1997; Angwafo, 1999). Dietary intake of animal or saturated fat is the environmental factor most consistently linked to significantly increased PC risk in previous studies, particularly among African-American men, though these associations appear too weak to explain more than a small fraction of observed racial-ethnic differences in PC risk (Whittemore *et al*., 1995; Hayes *et al*., 1999; Kolonel *et al*., 1999; Daniels et al., 2004), as also appears to be the case for other environmental/dietary factors examined such calcium, cruciferous vegetables, vitamin D, UV from sunlight, lycopene, and body size (Giovannucci *et al*., 1997, 1998; Cohen *et al*., 2000; Chan and Giovannucci, 2001a-b; Kristal and Lampe, 2002; Bodiwala et al., 2003). Because cooked-meat intake is positively associated with total saturated-fat intake (USDA, 1998), previous studies that focused on animal or saturated fat *per se* could have estimated effects due largely or entirely to meat-related HA intakes.

Consumption of cooked meats and associated HAs have been linked to increased risks of colorectal adenoma/adenocarcinoma and of cancer of the stomach, breast, lung and prostate (Mills *et al*., 1989; Shiffman and Felton, 1990; Gerhardsson de Verdier *et al*., 1991; Talamini *et al*., 1992; Lang *et al*., 1994; De Stefani *et al*., 1995; Ewings and Bowie, 1996; Probst-Hensch *et al*., 1997; Ward *et al*., 1997; Kampman *et al*., 1999; Norrish *et al*., 1999; Sinha *et al*., 1998a, 1999a-b; Zheng *et al*., 1998, 1999; Murtaugh et al., 2004), while fewer studies found no such associations (Lyon and Mahoney, 1988; Muscat and Wynder, 1994; Augustsson *et al*., 1999). Positive studies include those in which HA exposure was quantified adjusting for factors expected to determine HA intake—namely, meat type, consumption frequency, cooking method, and cooking doneness. A potential link between PhIP intake, in particular, and the elevated risk of PC experienced by African-American compared to Caucasian men is suggested by relatively greater levels of PhIP and its metabolites detected in urine sampled from the latter vs. the former group (Kidd *et al*., 1999), although urine analysis only provides a measure of PhIP exposure within 12-24 hours prior to sampling (Malfatti *et al*., 1999). Such a link is also supported by studies using meat- and cooking-method-specific HA-concentration estimates from multi-laboratory sets of experimental cooking data (Keating and Bogen, 2001) to assess intakes of PhIP and other HAs by >20,000 U.S. (including >3,000 African-American) participants in the nationwide, stratified, random-sample U.S. Continuing Survey of Food Intakes by Individuals (CSFII) (USDA, 1993, 1998). Analyzed by age-, sex, and race/ethnicity, these HA-exposure assessments found at all ages that African-American males consume on average at least twice as much PhIP (and total HA) per kg body weight per day as do U.S. Caucasian males, and that for both groups there is a significant positive (albeit small) correlation between estimated mean intakes of PhIP and those of saturated or total fat (Keating and Bogen, 2001; Bogen and Keating, 2001).

Although the study comparing HA intake (primarily from cooked lamb) and PC risk in New Zeeland men by Norrish *et al*. (1999) found no significant PhIP-related

associations involving PC, that study did report a significant association between PC risk and consumption of beefsteak by higher cooking-doneness category (2-sided $p_{trend}$ = 0.008). Several aspects of that study suggest it may have had limited power to assess potential associations between PC and HA or PhIP intake in the U.S. The incidence of PC in New Zeeland is only half that of Caucasian men in the U.S. (Hsing et al. 2000). Average meat (primarily lamb) consumption by subjects in the Norrish et al. study (~150 g/d) was well below that of U.S. Caucasian men (260 g/d) and very well below that of African-American men (304 g/d) (USDA, 1998; Table 10A). Moreover, the *minimum* PhIP intake (224 ng/d) in the *top* exposure quartile of Norrish et al. study subjects was well below the estimated *average* PhIP intakes by U.S. Caucasian (390 ng/d) and by African-American (600 ng/d) men (Bogen and Keating 2001).

PC Screening and PC

Periodic screening for plasma levels of Prostate-Specific Antigen (PSA) is useful for early detection of PC as well as benign prostatic hyperplasia (BPH) because PSA levels in serum are positively associated with age, prostate volume, and prostatic neoplastic disease (Carter *et al*., 1992; Oesterling *et al*., 1993; Etzioni *et al*., 1999). Significantly higher PSA levels are found in African-Americans than in whites, even after adjustment for age and prostate volume (in men without PC) and for PC grade and stage (in men with PC) (Abdalla *et al*., 1998a-b; Vijayakumar *et al*., 1998), due evidently to greater PSA secretion per unit prostate volume by African-American men (Fowler et al., 1999). Although a substantial fraction of “slightly” elevated PSA levels (between 4 and 10 ng/mL) is attributable to BPH or infection, “highly elevated” PSA levels (≥20 ng/mL) typically indicate a strong likelihood of localized or metastatic PC, with 80 to 90% positive predictivity and >99% specificity (Määttänen et al., 2001; Gerstenbluth et al., 2002; Smith et al., 2004). Likewise, a PSA measure < 4.0 ng/mL often considered within the “normal” range is actually associated with about at 15% of later PC diagnosis (Thompson et al., 2004).

## Report Body

**Objective/Hypotheses:** The study goal is to broaden the scope and power of a ground-breaking study of potential association between dietary HA exposure and screening indicators of PC risk in African-American men by adding a newer %fPSA test to the PSA/DRE protocol now being used. We hypothesize 1) that the added %fPSA test will increase PC detection in our study population, and 2) that the combined screening, follow-up diagnostic and dietary survey data obtained will reveal a positive association between estimated HA intake and screening and diagnostic indicators of increased PC risk in our African American study population.

**Specific aims:** Our aims are to 1) Add a newer %fPSA test to PSA and DRE screens being done in a current study of potential associations between HA and PC in Oakland, California, area African-American men. 2) Assess potential increased rate of PC

identified by including the %fPSA test with PSA and DRE results in light of clinical follow-up diagnoses obtained. 3) Use PSA-related and DRE test results, together with corresponding follow-up diagnostic and dietary survey data, to assess the potential association of HA-related exposure factors and increased PC risk in African-Americans.

**Study Design:** For aim 1, 400 participants are being solicited from an already-established network of churches, clinics and additional African-American community groups in the Oakland, CA, area. Detailed data on diet and meat consumption are being obtained by in-person interviews using established questionnaires, each followed by PSA-related and DRE screening tests, and follow-up diagnosis—a study design that avoids potential bias due to prior participant/investigator knowledge of PC status. Aims 2 and 3 will be accomplished by statistical data analysis, with aim 3 supported by incorporation of similar (other than %fPSA) data obtained for an additional 300 participants through our ongoing parallel NIH-funded study. Our focus on African-Americans provides needed study power, in view of the greater PC risk faced by this specific group.

## Progress in Relation to Work Plan

Work Plan Summary

A total of 392 participants were accrued in an ongoing NIH-funded study through December 2004 using dietary interviews, a digital rectal exam (DRE), and a standard prostate serum antigen (PSA) blood test. The DOD Prostate Cancer Research Program support has added a second PSA-related test— the "percent-free PSA" (%fPSA) test—to the protocol already applied to 82 additional participants as of December 2005, and will provide for 318 additional study participants through January of 2008, for whom blood samples will be used for both a PSA and a %fPSA test, over a two-year period, together with the dietary interview and DRE.

Delayed Receipt of Funds and HSRRB Approval of Human Subjects Protocol

Initiation of the study was delayed due to delayed receipt of DOD funds by Lawrence Livermore National Laboratory (LLNL). Due to contract language negotiations, funds arrived at LLNL approximately four months after the official start date of the project (Jan. 5, 2005). A further start-up delay occurred due to delayed receipt of U.S. Army Human Subjects Research Review Board (HSRRB) approval of the study human subjects protocol, which had already received approval by the other three Institutional Review Boards (IRBs) involved in this study (those of LLNL, the University of California San Francisco Medical School, and the Summit Alta Bates medical Center in Oakland, CA). A draft human subjects protocol was submitted to the HSRRB in December of 2004, but HSRRB approval was not obtained until mid-May of 2005.

In view of the delayed start-up of the project, a no-cost 6-month extension of this project (through June 31, 2008) will be requested *circa* August 2007 from the DOD grant manager for this project (Dr. L.C. Mishra, 301-619-7782).

Study Progress (indicated below in bold)

Note that, considering the ~6-month delay in start-up and anticipated corresponding performance-period extension, the time point of this reporting period in the study is approximately **Month 6** of an anticipated 3-year performance period.

Task 1. Add %fPSA test to the standard PSA blood test and DRE being done in a current study protocol to assess potential HA/PC associations in Oakland-area African American men (Months 1-27). **Initiated as of June 15, 2005.**

1.A. Obtain IRB approvals for modified study protocol (Months 1-3). Accomplishment of this task will be facilitated by the established protocol currently being used in the ongoing parallel NIH-funded study; approvals will be obtained for a modification of this protocol to add the %fPSA test to the PSA and DRE screens currently being used, to extend the study termination date by one year (through January 2008), and to increase the number of participants by 300 to a study total of 700. **Completed in May of 2005.**

1.B. Implement combined PSA-test protocol (PSA + %fPSA) for a total of 400 African American men screened at the Summit Alta-Bates MCEPC clinic in Oakland, California (Months 4-27). The %fPSA, PSA and DRE procedures to be used are all clinical procedures now performed routinely at the study clinic (Markstein Cancer Education and Prevention Center, Alta Bates Summit Medical Center, Oakland, CA). **Initiated as of June 15, 2005.**

1.C. We will interview study subjects and edit dietary questionnaires for all study subjects, to include:

1.C.1. 200 participants, comprising 100 DOD-funded participants plus 100 participants funded by NIH, all to be screened during Months 3-15 **A total of 82 (of 400) were completed as of December 31, 2005 (~16 more in January of 2006).**

1.C.2. 200 additional DOD-funded participants will be screened during Months 16-27. **Yet to be done.**

1.C.3. For all participants to be screened in this study, previously developed dietary survey questionnaires will be used in the same manner they are being used in our ongoing corresponding NIH-funded research study (see Questionnaires, Surveys & Clinical Protocols). **Initiated as of June 15, 2005.**

Task 2. Assess improvement in PC sensitivity and selectivity by addition of %fPSA test to the standard PSA test based on PSA-related test results and follow-up diagnoses in our African American study participants (Months 18-36). **All yet to be done.**

2.A. Data analysis methods to be used will be identical to those in use for the current related NIH-funded study (see Questionnaires, Surveys & Clinical Protocols)

2.B. Preliminary statistical analyses will be conducted during Months 18-27

2.C. Final statistical analyses will be conducted during Months 27-36

Task 3. Use Combined PSA-related test results, together with dietary survey data, to assess the potential association of HA-related exposure factors and increased PC risk in African Americans (Months 13-36).

3.A. Data analysis methods to be used will be identical to those in use for the current related NIH-funded study. **Analysis of initial data set (n = 392) obtained using the original protocol for NIH-funded work is described below. These results set the stage for follow-up analyses that will add new data involving %fPSA test results.**

3.B. Preliminary statistical analyses of the statistical validity of Study Hypotheses 1 and 2 will occur during Months 18-27. **Yet to be done.**

3.C. Final statistical analyses of the statistical validity of Study Hypotheses 1 and 2, as well as manuscript preparation, will occur during Months 27-36. **Yet to be done.**

3.D. We will also, as feasible, test the validity of Study Hypothesis 2 using combine DOD-funded data set (n = 400) with NIH-only-funded data set (n = 300), during Months 27-36. **Yet to be done, except for analysis of initial data set (n = 392) obtained using the original protocol for NIH-funded work, as described below. These results set the stage for follow-up analyses that will add new data involving %fPSA test results.**

<u>Preliminary Progress (Interim analysis of NIH-funded data set)</u>

In accordance with IRB-approved human subjects protocols, an ongoing clinic-based prospective study has enrolled male African-American volunteers from the Oakland, CA, area in accordance with the following inclusion criteria: (1) African-American men between 40 and 70 years old; (2) no previous PC diagnosis or medical condition preventing or interfering with study participation; and (3) written informed consent. Participation was facilitated by a $30 incentive payment, as well as by >10 yr of previous PC-related community outreach undertaken by the study clinic (the Markstein Cancer Education and Prevention Center at Summit Alta Bates Medical Center in Oakland, CA). After providing written informed consent, each participant

completed a PC-screening medical questionnaire, answered general and meat-/cooking-specific dietary questions, and was then provided free PC screening comprising a PSA blood test, and a digital-rectal exam by a board-certified urologist). General dietary intakes over the previous year were estimated using the Block-2000 questionnaire with portion-size arrays standardized food models to help each participant select portion sizes (Block et al., 1986). Meat-/cooking-specific dietary information pertaining to the previous year was obtained using an additional questionnaire including a validated set of standard meat-doneness descriptors and corresponding set of photographic meat-doneness descriptors (Alavanja et al., 1996; Sinha et al., 1998b-c). All dietary questionnaire were data were obtained by in-person interviews administered by trained dietary interviewers.

Combined survey data were used as previously described (Bogen and Keating, 2001) to estimate annual average dietary PhIP intake from all sources by each participant. Total and basal energy intake (in kcal per kg body weight) was estimated for each study participant by methods previously described (Bogen and Keating, 2001). Standard methods were used to assess the significance of linear associations and (where specifically mentioned) point-wise outliers therefrom, and to assess Pearson product-moment correlations (Draper and Smith, 1981; Selvin, 1995). Approximate significance of differences in mean HA-intake rates were compared by T-tests, using Welch's T-test in case of unequal variances as assessed by corresponding F-tests (Kendall and Stuart, 1979). Odds ratio (OR) and corresponding 95% confidence interval (CI) estimates were obtained by numerical maximum-likelihood procedures, and corresponding chi-square tests for trend, with or without adjustment for specified factors, was performed using standard methods (Breslow and Day, 1987). Difference between empirical cumulative mass functions (cmfs) was assessed by Kolmogorov 2-sample (K2S) test (Wilcox, 1997). All calculations were done using *Mathematica* 5.1® software (Wolfram, 1999).

Data on 392 African-American men who participated in this study are summarized in Table 1. Corresponding estimated average daily intakes of specific meats and of total PhIP are summarized in Figure 1. The empirical distribution of estimated daily intakes of PhIP from all meats ("total PhIP") (Figure 1, rightmost curve) has geometric and arithmetic mean values of 9.6 and 17 ng $kg^{-1}$ $d^{-1}$, respectively, and a geometric standard deviation of 3.34. Ratios of total to basal daily intake rates of energy per unit body weight ($E_{food}$:$E_{basal}$, unitless) estimated for this study population (not shown) have an arithmetic mean (±1 standard error of the mean) of 1.57 (± 0.046) not significantly different ($p = 0.51$) from the value of 1.6 expected for reference adult men (Bogen and Keating, 2001). All but approximately 11% of the inter-individual variance in PhIP intakes estimated using all meat- and cooking-method-specific doneness information was found to be explained by corresponding estimates conditioned on meat- and cooking-method-specific PhIP concentrations averaged over all participants, i.e., by factors (such as intake rates for specific meats cooked using specific methods) unrelated to differences in individually reported doneness preferences.

Estimated daily intakes of total PhIP were found to explain ~32% of observed inter-individual variance in corresponding estimated intakes of saturated fat per unit body

**Table 1.** Estimated mean HA intakes by all 21,858 CSFII participants who consumed HAs, by age group.

| Variable[a] | Value(s) | *n* | Mean |
|---|---|---|---|
| Age (y) | 39 to 50 | 115 | 47.2 |
| | 51 to 60 | 216 | 54.6 |
| | 61 to 70 | 61 | 63.6 |
| | All | 392 | 53.9 |
| Weight (kg) | 54.4 to 163 | 392 | 85.7[c] |
| BMI (kg cm$^{-2}$) | <30 | 317 | 25.2 |
| | ≥30 | 75 | 33.9 |
| | 18.4 to 46.0 | 392 | 26.9 |
| PSA (ng/mL) | < 2 | 328 | 0.79 |
| | 2 to <4 | 42 | 2.7 |
| | 4 to <10 | 10 | 5.5 |
| | 10 to <20 | 7 | 14 |
| | ≥20 | 5 | 47 |
| | | PSA<4 | PSA≥4[a] |
| DRE | Normal | 233 | 15 |
| | BPH | 108 | 4 |
| | Suspicious[a] | 12 | 3 |
| Family history[b] | No | 333 | 15 |
| | Yes | 37 | 7 |

**Notes**

[a] BMI = body mass index; DRE = digital rectal exam; Suspicious = abnormal DRE result leading to medical referral; Normal = no urinary, BPH or other symptoms. Each abnormal PSA result (≥4.0 ng/mL) triggered referral for medical follow-up.

[b] Family history = self reported brother(s) and/or father diagnosed with prostate cancer. Association of positive family history with PSA ≥ 4 ng/mL is significantly positive by 2-tail Fisher exact test (p = 0.013).

[c] Weight median (interquartile range) = 83.9 (74.8 to 95.3) kg.

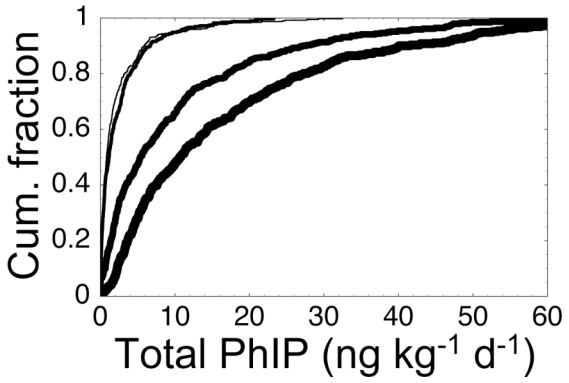


**Figure 1.** Cumulative distributions of estimated PhIP intake by meat type for the study population of 392 Oakland, CA-area African-American men. Curves with increasing boldness denote the following meat types (and corresponding % of total estimated intake): burger (15%), other beef (14%), chicken (61%), and total (100%).

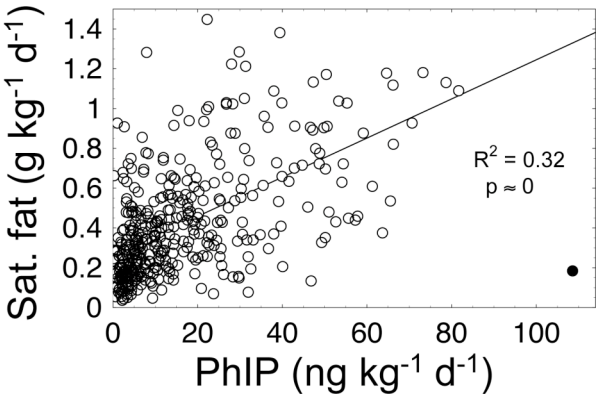


**Figure 2.** Saturated fat (g/kg-d) intake estimated by FFQ vs. PhIP intake estimated by the LLNL Meat questionnaire for 392 Oakland, CA-area African-American men (open points). Linear regression shown (solid line) indicates a significantly positive association. A single point (closed circle) determined to be an outlier ($p < 10^{-5}$ by F test) was excluded from the regression

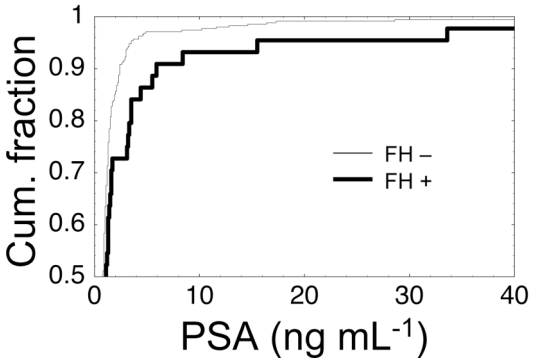


**Figure 3.** Cumulative distributions of PSA measured in 392 Oakland, CA-area African-American men who did (FH +), vs. who who did not (FH –), report a father and/or brothers diagnosed with prostate cancer.

**Table 2**. Association of PhIP intake with elevated PSA in African-American men ≥ 55 years old.

| Adjustment or restriction[a] . Ave. PhIP intake[a] PR[a] (ng kg$^{-1}$ d$^{-1}$) | PSA ≥ 20 ng/mL[b] *m* | *n* | OR[a] | 95%LCL[a] | 95%UCL[a] | $p_{trend}$[a] |
|---|---|---|---|---|---|---|
| All data | | | | | | |
| 0-50: 4.6 | 0 | 83 | 1 | | | |
| >50-70: 14.4 | 1 | 33 | 7.6 | 0.47 | 200 | |
| >70-85: 24.0 | 0 | 25 | – | – | – | |
| >85-100: 47.7 | 4 | 26 | 32. | 3.2 | 720 | 0.00020 |
| Adj. for FH | | | | | | |
| (same as above) | (*m*, *n*, ORs, LCLs and UCLs approx. unchanged[c]) | | | | | 0.00039 |
| FH – | | | | | | |
| 0-50: 4.6 | 0 | 76 | 1 | | | |
| >50-70: 14.4 | 1 | 27 | 8.5 | 0.52 | 220 | |
| >70-85: 24.0 | 0 | 22 | – | – | – | |
| >85-100: 47.7 | 2 | 22 | 18. | 1.5 | 430 | 0.016 |
| FH + | | | | | | |
| 0-50: 4.6 | 0 | 22 | 1 | | | |
| >50-70: 14.4 | 0 | 9 | – | – | – | |
| >70-85: 24.0 | 0 | 6 | – | – | – | |
| >85-100: 47.7 | 2 | 7 | 18. | 1.4 | 540 | 0.0067 |
| Adj. for SatFat | | | | | | |
| 0-50: 4.6 | 0 | 83 | 1 | | | |
| >50-70: 14.4 | 1 | 33 | 4.1 | 0.25 | 110 | |
| >70-85: 24.0 | 0 | 25 | – | – | – | |
| >85-100: 47.7 | 4 | 26 | 32. | 3.2 | 720 | 0.0024 |
| Adj. for KCAL | | | | | | |
| 0-50: 4.6 | 0 | 83 | 1 | | | |
| >50-70: 14.4 | 1 | 33 | – | – | – | |
| >70-85: 24.0 | 0 | 25 | 4.0 | 0.24 | 110 | |
| >85-100: 47.7 | 4 | 26 | 32. | 3.2 | 720 | 0.0011 |

[a] PR = percentile range; FH = family history (self reported brother/s and/or father diagnosed with prostate cancer; SatFat = daily saturated fat intake per kg body weight; KCAL = total energy intake per kg body weight. Trend analyses adjusting for SatFat or KCAL were each done using the adjustment variable dichotomized at its median value.

[b] *m* = number with PSA ≥ 20 ng/mL among *n* total participants included in the analysis.

[c] At mean PhIP-intake level 14.4 ng kg$^{-1}$ d$^{-1}$, OR (95%CL) = 8.5 (0.52, 220).

weight (Figure 2). Similar of greater levels of positive correlation were observed between estimated total PhIP intake and energy-intake ratio ($E_{food}$:$E_{basal}$) ($R^2$ = 0.26), total energy intake ($E_{food}$) ($R^2$ = 0.27), total meat intake (g $kg^{-1}$ $d^{-1}$) ($R^2$ = 0.68), and between estimated intakes of total energy and saturated fat ($R^2$ = 0.84) and $E_{food}$:$E_{basal}$ ($R^2$ = 0.97). PSA measures were found to have a weak positive association with participant age that attained statistical significance for all measures < 4 ng/mL ($R^2$ = 0.051, p = 0.000012), but not for all measures ≥ 4 ng/mL ($R^2$ = 0.035, p = 0.41). All PSA measures ≥ 20 ng/mL were obtained for participants between 55 and 65 years old.

Figure 3 summarizes PSA measures among participants reporting vs. not reporting a close relation (a father and/or brother) with PC. PSA measures were found to be significantly greater among those reporting such a family history (p = 0.023 by K2S test), particularly when the comparison is restricted to the upper quartile of PSA measures in each family-history category (p = $1.9 \times 10^{-6}$ by K2S test). Although positive family history was found to be significantly positively associated with elevated PSA defined as any measure ≥ 4 ng/mL (p = 0.013 by 2-tail Fisher exact test, Table 1), this was not the case using the following alternative elevated-PSA criteria: ≥ (vs. <) 10 ng/mL (p = 0.28), ≥ (vs. <) 15 ng/mL (p = 0.099), ≥ (vs. <) 20 ng/mL (p = 0.20), or ≥ 20 vs. < 4 ng/mL (p = 0.17).

Because estimated PhIP intakes were found to have a highly skewed distribution (Figure 1), association between PhIP intake and highly elevated PSA (≥ 20 ng/mL) status was investigated using PhIP-intake bin boundaries defined by the $50^{th}$, $70^{th}$, and $85^{th}$ percentile values of the empirical intake distribution. Thus categorized, PhIP-intake level was found to be significantly positively associated with highly elevated PSA status, compared to this status among participants in the lowest half of estimated PhIP intakes, with or without single-variable adjustment for father/brother family history of PC, saturated fat intake, or total energy (corresponding p-values for trend of $p_{trend}$ = 0.014 for SatFat adjustment, $p_{trend} \leq 0.003$ for all other tests). As mentioned, all highly elevated PhIP measures occurred in men within a fairly narrow age range (55 to 65 years old). In just the men ≥ 55 years old, PhIP-intake level was found to be even more significantly positively associated with highly elevated PSA status, compared to this status among this subset of participants who also were in the lowest half of estimated PhIP intakes, with or without single-variable adjustment for father/brother family history of PC, saturated fat intake, or total energy (Table 2). In particular, this association among men in the highest 15% compared to those in the bottom 50% of estimated PhIP intakes was found to have a maximum-likelihood odds ratio (and corresponding 95% confidence limits) of 32 (3.6, 720), with a corresponding p-value for trend of $p_{trend}$ = 0.00020. Adjustment for father/brother family history of PC, saturated fat intake, or total energy yielded identical odds-ratio estimates, and only slightly greater estimates of $p_{trend}$ (Table 2).

A generally similar significant pattern of positive association was also observed between estimated PhIP intake and men with highly elevated PC risk defined as either PSA ≥ 20 ng/mL or a “suspicious” abnormal DRE result leading to medical referral (data not shown). Such suspicious DRE results were obtained among a ~4-fold greater fraction (3

of 12) participants with mildly elevated PSA (≥ 4.0 ng/mL) than among (12 of 370 of) those with PSA < 4.0 ng/mL—a difference that is not statistically significant (p = 0.11 by 2-tail Fisher exact test) perhaps due to study size. Suspicious DRE results were not obtained for any participant with a PSA measure ≥ 20 ng/mL. Separate trend analyses done to assess for positive association between highly elevated PSA and (either quintiles, or {50, 70, 85}th percentile intervals of) either saturated fat intake, total energy intake, or body mass index, did not yield statistically significant trend-test results with or without adjustment for PhIP intake ($p_{trend} > 0.10$ for each of 12 separate tests).

The interim data obtained from the ongoing study described are consistent with the hypothesis that a PSA- related, and a combined PSA- and DRE-related, screening indicator of highly elevated PC risk is significantly positively associated with estimated dietary exposure to PhIP. Although this conclusion remains tentative in view of the fairly small number of men involved in this prospective study so far, it is supported by the consistency of the pattern of results found, their level of statistical significance, and the significant positive association observed between highly elevated PSA and a positive father/brother history of PC consistent with previous studies that clearly have linked this factor to elevated PC risk (see Introduction).

An apparent contradiction between results obtained in this study and those of that comparing HA intake (primarily from cooked lamb) and PC risk in New Zeeland men (including 317 PC cases and 480 age-matched controls) studied by Norrish et al. (1999)—who found no significant PhIP-related associations involving PC—is easily explained. The lower bound on individual-average daily PhIP intake (224 ng/d) that study used in its top exposure quartile that was about 12-fold lower than the lower bound (of about 2680 ng/d) of the top 15% (the highest category) of PhIP exposures estimated for the East San Francisco Bay area African-American men in the present study. If the dose-response for PhIP-induced elevation of PC risk were assumed to be linear no-threshold, linear extrapolation from significantly elevated OR estimates ≥ 2.2 or 3.2 obtained in the present study down to PhIP exposure levels used in the Norrish et al. (1999) study would require the latter study to be able to detect elevated OR values as low as about 1.2 or 1.3 in order to have detected a significant PhIP-related association with 95% confidence at 80% statistical power. In contrast, exact evaluation of non-central hypergeometric distributions (Zelterman, 1999) corresponding to the Norrish et al. (1999) study design indicate it could only detect an OR as low as about 1.8 with 95% confidence at 80% power for comparisons involving its lowest vs. highest exposure quartiles. Moreover, the empirical relation between mutagenic and carcinogenic potencies of genotoxic rodent-carcinogen chemicals, such as PhIP, suggests that the low-dose dose-response for cancer induction by such chemicals is likely to be sublinear in general (Bogen, 1993), which implies that the Norrish et al. (1999) design had even less power to detect elevated PC risk due to PhIP exposure levels studied.

Ideally, a prospective study accumulates definitive diagnostic data together with data on exposure- or treatment-related variables of interest. A key limitation to interpretation of data obtained in this study so far is thus that, despite ongoing work to obtain

corresponding follow-up diagnostic data, a PC diagnosis is not yet (and for some, may never be) available for all participants who received either positive or highly elevated PC screening results or who received a "suspicious" DRE leading to medical referral. Compared to a case-control design, the prospective design used in this ongoing study has the advantage of being double blind, insofar as PC screening results analyzed are in each case known neither by the participant nor by study investigators until after each participant provides dietary survey data. This design eliminates potential bias (e.g., in participants' self-reported cooking preferences) associated with knowledge of PC-screening results or PC status, which is important in view of evidence that prior knowledge of cancer-related status can affect dietary recall and so induce significant differential misclassification (Wilkens et al., 1992).

Summary of Interim Results

To investigate the hypothesis that PhIP exposure increases PC risk, an ongoing prospective clinic-based study has compared PC-screening outcomes with survey-based estimates of dietary PhIP intake by 40- to 70-year-old African-American men in the Oakland, CA, area. Participants with no prior PC diagnosis, recruited through a cancer education center/screening clinic, complete food-frequency and meat-cooking/consumption questionnaires, and have a prostate serum antigen (PSA) test and digital-rectal exam. Preliminary results for 392 participants indicate that mean (±1 SD) daily intake of PhIP, the major HA found in cooked meats, in this group is 17 (±17) ng $kg^{-1}$ $d^{-1}$, which is ~2-fold (and ~3-fold) greater than a national estimate of mean PhIP intake for African-American (and white U.S.) men of similar age. In the present study, estimated PhIP intakes were found to be attributable mostly (61%) to chicken and positively associated ($R^2 = 0.32$, $p \sim 0$) with estimated saturated fat (SF) intake (previously hypothesized to be a key environmental PC-risk factor). A conditional maximum-likelihood odds ratio, OR, (and 95% confidence limits) of 32 (3.2, 720) for highly elevated PSA ≥20 ng/mL was observed in the highest 15% compared to the lower 50% of estimated daily PhIP intakes (≥30 vs. ≤10 ng $kg^{-1}$ $d^{-1}$) by men 55+ years old, with a p-value ($p_{trend}$) of 0.00020 for a corresponding chi-square test for trend done across these and two additional PhIP-intake groups. This positive trend persisted after separate adjustments for self-reported family (brother or father) history of PC (FH), SF intake, and energy intake ($p_{trend}$ = 0.00039, 0.0024, and 0.0011, respectively). PSA measures were found (by Kolmogorov 2-sample tests) to be significantly higher in African-American men reporting a positive FH in this study ($p = 0.023$), particularly for those among the highest PSA-measure quartile in each FH group ($p < 10^{-5}$). These preliminary results are consistent with a positive association between PhIP intake and highly elevated PSA levels, supporting the hypothesis that diet and food preparation interventions may help reduce PC risk in African-American and perhaps other groups. Current and planned related work involving additional study participants, from whom an additional PC screening measure (%fPSA) is being obtained, will increase the power of this study to detect and investigate PhIP-related effects on PC risk in the African-American men studied.

## Key Research Accomplishments

The NIH-funded P01 work that set the stage for the present expanded study has accomplished the two specific aims it sought to address. We successfully applied methods for estimating HA concentrations in cooked meats based on individually expressed data on meat-specific intakes, cooking methods and doneness preferences to estimate daily PhIP intakes, and we have found these intake estimates to be positively associated with screening indicators of highly elevated PC risk in a prospective clinic-based PC screening study involving nearly 400 African-American men in the San Francisco East Bay area. The observed positive association, which was most significant among men 55 to 70 years of age ($p_{trend} = 0.00020$), remained statistically significant after adjustments for saturated fat intake, total energy intake and self-reported father/brother history of PC.

Future work will continue to accrue participants using the same basic study design, except insofar as to expand the screening indicators used to predict PC status to include the %fPSA test, and to assess whether PhIP-related associations pertain to combined screening indicator data using a larger study population. In so doing, this study will continue to help define the potential value of improved screening and dietary/behavioral intervention to reduce PC risk, namely, prevention of PhIP intake by avoiding overcooked meats.

## Reportable Outcomes

Abstracts/Posters

Bogen, K, W Baker, J Chan, D Nelson, E Holly, G Keating, L Paine, and J Felton. 2005. Prostate cancer screening and dietary HA exposure in African-Americans. UCRL-POST-211476. Poster presented at the University of California Davis Health System Future Fair, May 5, 2005, UC Davis Medical Center, Sacramento, CA.

KT Bogen, GA Keating, EA Holly, J Chan, L Paine, EL Simms, DO Nelson, and J Felton. 2005. Prostate Serum Antigen levels and dietary heterocyclic amines in African Americans: A prospective clinic-based study. Abstract of poster accepted for presentation at the 97th Annual Meeting of the American Assoc. for Cancer Research, April 1-5, 2006, Washington, DC. UCRL-ABS-217085. Lawrence Livermore National Laboratory, Livermore, CA.

Manuscripts

Keating, GA, and K Bogen. 2006. Development of a meat frequency questionnaire for use in diet and cancer studies. Submitted to *J. Am. Dietetic Assoc*. UCRL-JRNL-208025, Lawrence Livermore National Laboratory, Livermore, CA.

Bogen KT, GA Keating, JM Chan, EA Holly LJ Paine, EL Simms, and DO Nelson. 2006. Highly elevated PSA and dietary PhIP intake by African Americans: Interim results from a prospective clinic-based study. Prepared for submittal to *J. Expos. Anal. Environ. Epidemiol*. [included in the Appendix of this report]

## Conclusion

Prostate cancer (PC) is the second leading cause of male U.S. cancer deaths, with African-Americans having the highest rate of PC mortality worldwide, as well as more abnormal results from screening tests that correlate with current or eventual PC. A 3-year prospective clinic-based study is studying the performance of current (PSA and DRE) vs. (% free PSA) clinical biomarkers of PC risk in 400 African-American men 50 to 70 years of age who undergo PC screening in Oakland, CA (East Bay San Francisco area), as well as possible association of PC screening results for these men with their dietary exposures to the cancer-causing heterocyclic amine, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) that forms when meat is cooked. This study expands an ongoing NIH-funded study (by the same research team) to add a new %-free-PSA test, results of which will be compared with PSA/ DRE results and PhIP exposures estimated by dietary interviews. For 392 men studied under the NIH protocol, an odds ratio (95% CL) of 32 (3.2, 720) for highly elevated PSA (≥20 ng/mL) was observed in the highest 15% vs. the lower 50% of estimated daily PhIP intakes. Approximately 100 additional men have completed participation in the expanded NIH/DOD-supported study.

Future work will continue to accrue participants in this study, expand the screening indicators used to predict PC status to include the %fPSA test, and to assess whether PhIP-related associations pertain to combined screening indicator data using a larger study population. In so doing, this study will continue to help define the potential value of improved screening and dietary/behavioral intervention to reduce PC risk, namely, prevention of PhIP intake by avoiding overcooked meats.

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# Appendix

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# Highly elevated PSA and dietary PhIP intake by African Americans: Interim results from a prospective clinic-based study

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*Abbreviations (CAS #)*: HA = heterocyclic amine, PC = prostate cancer, PhIP = 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (105650-23-5).

# ABSTRACT

African American (AA) men, who compared to Caucasians die nearly twice as much from prostate cancer (PC), also take in about twice as much of the predominant U.S. dietary heterocyclic amine, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), which occurs primarily in well-cooked chicken and beef. To investigate the hypothesis that PhIP exposure increases PC risk, an ongoing prospective clinic-based study has compared PC-screening outcomes with survey-based estimates of dietary PhIP intake by 40- to 70-year-old AA men in the Oakland, CA, area. Participants with no prior PC diagnosis, recruited through a cancer education center/screening clinic, complete food-frequency and meat-cooking/consumption questionnaires, and have a prostate serum antigen (PSA) test and digital-rectal exam. Preliminary results for 392 participants indicate that mean (±1 SD) daily intake of PhIP, the major HA found in cooked meats, in this group is 17 (±17) ng $kg^{-1}$ $d^{-1}$, which is ~2-fold (and ~3-fold) greater than a national estimate of mean PhIP intake for AA (and white U.S.) men of similar age. In the present study, estimated PhIP intakes were found to be attributable mostly (61%) to chicken and positively associated ($R^2$ = 0.32, p ~ 0) with estimated saturated fat (SF) intake (previously hypothesized to be a key environmental PC-risk factor). A maximum-likelihood odds ratio, OR, (and 95% confidence limits) of 32 (3.2, 720) for highly elevated PSA ≥20 ng/mL was observed in the highest 15% compared to the lower 50% of estimated daily PhIP intakes (≥30 vs. ≤10 ng $kg^{-1}$ $d^{-1}$) by men 55+ years old, with a p-value ($p_{trend}$) of 0.00020 for a corresponding chi-square test for trend done across these and two additional PhIP-intake groups. This positive trend persisted after separate adjustments for self-reported family (brother or father) history of PC (FH), SF intake, and energy intake ($p_{trend}$ = 0.00039, 0.0024, and 0.0011, respectively). PSA measures were found (by Kolmogorov 2-sample tests) to be significantly higher in AA men reporting a positive FH in this study (p = 0.023), particularly for those among the highest PSA-measure quartile in each FH group ($p < 10^{-5}$). These preliminary results are consistent with a positive association between PhIP intake and highly elevated PSA levels, supporting the hypothesis that diet and food preparation interventions may help reduce PC risk in AA and perhaps other groups.

**Key Words:** Age, cancer, cooking, meat mutagens, energy, meat, prostate, race, sex, variability

## Introduction

Heterocyclic amines (HAs) are potent mutagens formed in meats, chicken and fish as it is cooked to higher-doneness levels by heat-intensive cooking methods (Thompson *et al*., 1987; Keating et al., 1999, 2000). HAs also cause cancer at a variety of sites in multiple bioassay animal species/strains/sexes, as well as at multiple sites within many of species/strains/sexes tested (Bogen, 1994). A predominant HA found in cooked and particularly in well-done chicken and beef is 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) (Felton et al., 1984, 1986; Felton and Knize, 1990a-b; Sinha et al., 1995). Dietary exposure to PhIP has been shown to induce colon, intestinal and mammary adenocarcinomas in rats (Ohgaki et al., 1986; Ochiai et al., 1991; Ito et al., 1991; Ito et al., 1997; Ghoshal et al., 1994), as well as prostate cancer in rats (Shirai et al., 1997,1999). HAs are metabolically activated by P450 and N-acetyltransferase enzymes to activated forms that bind covalently to (among other targets) DNA in tissues (including in prostate) where HAs induce cancer in rats (Thorgeirsson et al., 1983; Thorgeirsson, 1984; Rosenkranz and Mermeistein, 1985; Sato et al., 1986; Kato and Yamazoe, 1987; Snyderwine and Battula, 1989; McManus et al., 1990; Turesky et al., 1991; Davis et al., 1993; Kaderlik et al., 1994a-b; Takahashi et al., 1998; Schut and Snyderwine, 1999; Gooderham et al., 2002). In male *lacI* trangenic rats, a diet containing 200 ppm PhIP was shown to be highly mutagenic in prostate tissue (Stuart *et al*., 2000). In cultured human prostate tissue and primary prostate cells, PhIP is metabolically activated to mutagenic forms that covalently bind to and damage DNA (Williams et al., 2000; Lawson and Kolar, 2002; Martin et al., 2002; Di Paolo et al., 2005).

In the U.S., prostate cancer (PC) is a leading cause of cancer death among men, with African Americans having the highest age-specific prostate cancer rate in the world, and a >2-fold higher rate of mortality for PC than white men in the U.S.(Miller *et al*., 1996; Robbins *et al*., 1998; Hsing *et al*., 2000). Although family (particularly father/brother) history of PC is clearly linked to substantially elevated PC risk (Schuurman *et al*., 1999; Hemminki and Czene, 2002; Zeegers et al., 2003; Hemminki and Chen, 2005) and a human-PC-specific chromosome translocation has been identified (Tomlins et al., 2005), there is (as for most other cancers) no evidence for a predominant heritable factor for PC. Racial-ethnic differences in levels of testosterone-related hormones and related genetic controls on hormone-induced prostate growth have been hypothesized to explain or contribute to corresponding racial-ethnic differences in PC risk, but their role remains unclear (Ross *et al*., 1998; Pettaway, 1999; Hsing, 2001). Even if hormonally mediated background processes do affect PC risk, dietary exposures to genotoxic

HA carcinogens may act independently or interact synergistically with such hormonal processes to further modify PC risk.

Substantial geographical variations in PC incidence indicate the importance of one or more dietary or other environmental factors (Minami et al., 1993; Mettlin, 1997; Angwafo, 1999). Dietary intake of animal or saturated fat is the environmental factor most consistently linked to significantly increased PC risk in previous studies, particularly among African-American men, though these associations appear too weak to explain more than a small fraction of observed racial-ethnic differences in PC risk (Whittemore *et al*., 1995; Hayes *et al*., 1999; Kolonel *et al*., 1999; Daniels et al., 2004), as also appears to be the case for other environmental/dietary factors examined such calcium, cruciferous vegetables, vitamin D, UV from sunlight, lycopene, and body size (Giovannucci *et al*., 1997, 1998; Cohen *et al*., 2000; Chan and Giovannucci, 2001a-b; Kristal and Lampe, 2002; Bodiwala et al., 2003). Because cooked-meat intake is positively associated with total saturated-fat intake (USDA, 1998), previous studies that focused on animal or saturated fat *per se* could have estimated effects due largely or entirely to meat-related HA intakes.

Consumption of cooked meats and associated HAs have been linked to increased risks of colorectal adenoma/adenocarcinoma and of cancer of the stomach, breast, lung and prostate (Mills *et al*., 1989; Shiffman and Felton, 1990; Gerhardsson de Verdier *et al*., 1991; Talamini *et al*., 1992; Lang *et al*., 1994; De Stefani *et al*., 1995; Ewings and Bowie, 1996; Probst-Hensch *et al*., 1997; Ward *et al*., 1997; Kampman *et al*., 1999; Norrish *et al*., 1999; Sinha *et al*., 1998a, 1999a-b; Zheng *et al*., 1998, 1999; Murtaugh et al., 2004), while fewer studies found no such associations (Lyon and Mahoney, 1988; Muscat and Wynder, 1994; Augustsson *et al*., 1999). Positive studies include those in which HA exposure was quantified adjusting for factors expected to determine HA intake—namely, meat type, consumption frequency, cooking method, and cooking doneness. A potential link between PhIP intake, in particular, and the elevated risk of PC experienced by African-American compared to Caucasian men is suggested by relatively greater levels of PhIP and its metabolites detected in urine sampled from the latter vs. the former group (Kidd *et al*., 1999), although urine analysis only provides a measure of PhIP exposure within 12-24 hours prior to sampling (Malfatti *et al*., 1999). Such a link is also supported by studies using meat- and cooking-method-specific HA-concentration estimates from multi-laboratory sets of experimental cooking data (Keating and Bogen, 2001) to assess intakes of PhIP and other HAs by >20,000 U.S. (including >3,000 African-American) participants in the nationwide, stratified, random-sample U.S. Continuing Survey of Food Intakes by Individuals (CSFII) (USDA,

1993, 1998). Analyzed by age-, sex, and race/ethnicity, these HA-exposure assessments found at all ages that African-American males consume on average at least twice as much PhIP (and total HA) per kg body weight per day as do U.S. Caucasian males, and that for both groups there is a significant positive (albeit small) correlation between estimated mean intakes of PhIP and those of saturated or total fat (Keating and Bogen, 2001; Bogen and Keating, 2001).

Although the study comparing HA intake (primarily from cooked lamb) and PC risk in New Zeeland men by Norrish *et al.* (1999) found no significant PhIP-related associations involving PC, that study did report a significant association between PC risk and consumption of beefsteak by higher cooking-doneness category (2-sided $p_{trend}$ = 0.008). Several aspects of that study suggest it may have had limited power to assess potential associations between PC and HA or PhIP intake in the U.S. The incidence of PC in New Zeeland is only half that of Caucasian men in the U.S. (Hsing et al. 2000). Average meat (primarily lamb) consumption by subjects in the Norrish et al. study (~150 g/d) was well below that of U.S. Caucasian men (260 g/d) and very well below that of African-American men (304 g/d) (USDA, 1998; Table 10A). Moreover, the *minimum* PhIP intake (224 ng/d) in the *top* exposure quartile of Norrish et al. study subjects was well below the estimated *average* PhIP intakes by U.S. Caucasian (390 ng/d) and by African-American (600 ng/d) men (Bogen and Keating 2001).

Periodic screening for plasma levels of Prostate-Specific Antigen (PSA) is useful for early detection of PC as well as benign prostatic hyperplasia (BPH) because PSA levels in serum are positively associated with age, prostate volume, and prostatic neoplastic disease (Carter *et al.*, 1992; Oesterling *et al.*, 1993; Etzioni *et al.*, 1999). Significantly higher PSA levels are found in African-Americans than in whites, even after adjustment for age and prostate volume (in men without PC) and for PC grade and stage (in men with PC) (Abdalla *et al.*, 1998a-b; Vijayakumar *et al.*, 1998), due evidently to greater PSA secretion per unit prostate volume by African-American men (Fowler et al., 1999). Although a substantial fraction of "slightly" elevated PSA levels (between 4 and 10 ng/mL) is attributable to BPH or infection, "highly elevated" PSA levels (≥20 ng/mL) typically indicate a strong likelihood of localized or metastatic PC, with 80 to 90% positive predictivity and >99% specificity (Määttänen et al., 2001; Gerstenbluth et al., 2002; Smith et al., 2004). Likewise, a PSA measure < 4.0 ng/mL often considered within the "normal" range is actually associated with about at 15% of later PC diagnosis (Thompson et al., 2004). The present study was conducted to assess the association between highly elevated PSA and estimated dietary exposure to PhIP.

## Methods

*Study design.* In accordance with IRB-approved human subjects protocols, an ongoing clinic-based prospective study has enrolled male African-American volunteers from the Oakland, CA, area in accordance with the following inclusion criteria: (1) African-American men between 40 and 70 years old; (2) no previous PC diagnosis or medical condition preventing or interfering with study participation; and (3) written informed consent. Participation was facilitated by a $30 incentive payment, as well as by >10 yr of previous PC-related community outreach undertaken by the study clinic (the Markstein Cancer Education and Prevention Center at Summit Alta Bates Medical Center in Oakland, CA). After providing written informed consent, each participant completed a PC-screening medical questionnaire, answered general and meat-/cooking-specific dietary questions, and was then provided free PC screening comprising a PSA blood test, and a digital-rectal exam by a board-certified urologist). General dietary intakes over the previous year were estimated using the Block-2000 questionnaire with portion-size arrays standardized food models to help each participant select portion sizes (Block et al., 1986). Meat-/cooking-specific dietary information pertaining to the previous year was obtained using an additional questionnaire including a validated set of standard meat-doneness descriptors and corresponding set of photographic meat-doneness descriptors (Alavanja et al., 1996; Sinha et al., 1998b-c). All dietary questionnaire were data were obtained by in-person interviews administered by trained dietary interviewers.

*Data Analysis*. Combined survey data were used as previously described (Bogen and Keating, 2001) to estimate annual average dietary PhIP intake from all sources by each participant. Total and basal energy intake (in kcal per kg body weight) was estimated for each study participant by methods previously described (Bogen and Keating, 2001). Standard methods were used to assess the significance of linear associations and (where specifically mentioned) point-wise outliers therefrom, and to assess Pearson product-moment correlations (Draper and Smith, 1981; Selvin, 1995). Approximate significance of differences in mean HA-intake rates were compared by T-tests, using Welch's T-test in case of unequal variances as assessed by corresponding F-tests (Kendall and Stuart, 1979). Odds ratio (OR) and corresponding 95% confidence interval (CI) estimates were obtained by numerical maximum-likelihood procedures, and corresponding chi-square tests for trend, with or without adjustment for specified factors, was performed using standard methods (Breslow and Day, 1987). Difference between empirical cumulative mass functions (cmfs) was assessed by Kolmogorov 2-sample (K2S) test (Wilcox, 1997). Significance p-values $\leq 10^{-10}$ are reported as being $\approx 0$. All calculations were done using *Mathematica* 5.1® software (Wolfram, 1999).

## Results

Data on 392 African-American men who participated in this study are summarized in Table 1. Corresponding estimated average daily intakes of specific meats and of total PhIP are summarized in Table 2 and Figure 1. The empirical distribution of estimated daily intakes of PhIP from all meats ("total PhIP") (Figure 1, rightmost curve) has geometric and arithmetic mean values of 9.6 and 17 ng $kg^{-1}$ $d^{-1}$, respectively, and a geometric standard deviation of 3.34. Ratios of total to basal daily intake rates of energy per unit body weight ($E_{food}$:$E_{basal}$, unitless) estimated for this study population (Figure 2) have an arithmetic mean (±1 standard error of the mean) of 1.57 (± 0.046) not significantly different ($p = 0.51$) from the value of 1.6 expected for reference adult men (Bogen and Keating, 2001). All but approximately 11% of the inter-individual variance in PhIP intakes estimated using all meat- and cooking-method-specific doneness information was found to be explained by corresponding estimates conditioned on meat- and cooking-method-specific PhIP concentrations averaged over all participants, i.e., by factors (such as intake rates for specific meats cooked using specific methods) unrelated to differences in individually reported doneness preferences (Figure 3).

Estimated daily intakes of total PhIP were found to explain ~32% of observed inter-individual variance in corresponding estimated intakes of saturated fat per unit body weight (Figure 4). Similar of greater levels of positive correlation were observed between estimated total PhIP intake and energy-intake ratio ($E_{food}$:$E_{basal}$) ($R^2 = 0.26$), total energy intake ($E_{food}$) ($R^2 = 0.27$), total meat intake (g $kg^{-1}$ $d^{-1}$) ($R^2 = 0.68$), and between estimated intakes of total energy and saturated fat ($R^2 = 0.84$) and $E_{food}$:$E_{basal}$ ($R^2 = 0.97$).

PSA measures were found to have a weak positive association with participant age that attained statistical significance for all measures < 4 ng/mL ($R^2 = 0.051$, $p = 0.000012$), but not for all measures ≥ 4 ng/mL ($R^2 = 0.035$, $p = 0.41$). All PSA measures ≥ 20 ng/mL were obtained for participants between 55 and 65 years old.

Figure 5 summarizes PSA measures among participants reporting vs. not reporting a close relation (a father and/or brother) with PC. PSA measures were found to be significantly greater among those reporting such a family history ($p = 0.023$ by K2S test), particularly when the comparison is restricted to the upper quartile of PSA measures in each family-history category ($p = 1.9 \times 10^{-6}$ by K2S test). Although positive family history was found to be significantly positively associated with elevated PSA defined as any measure ≥ 4 ng/mL ($p = 0.013$ by 2-tail Fisher exact test, Table 1), this was not the case using the following alternative elevated-PSA criteria: ≥ (vs. <) 10 ng/mL ($p = 0.28$), ≥ (vs. <) 15 ng/mL ($p = 0.099$), ≥ (vs. <) 20 ng/mL ($p = 0.20$), or ≥ 20 vs. < 4 ng/mL ($p = 0.17$).

Because estimated PhIP intakes were found to have a highly skewed distribution (Figure 1), association between PhIP intake and highly elevated PSA (≥ 20 ng/mL) status was investigated using PhIP-intake bin boundaries defined by the 50$^{th}$, 70$^{th}$, and 85$^{th}$ percentile values of the empirical intake distribution. Thus categorized, PhIP-intake level was found to be significantly positively associated with highly elevated PSA status, compared to this status among participants in the lowest half of estimated PhIP intakes, with or without single-variable adjustment for father/brother family history of PC, saturated fat intake, or total energy (Table 3). As mentioned, all highly elevated PhIP measures occurred in men within a fairly narrow age range (55 to 65 years old). In just the men ≥ 55 years old, PhIP-intake level was found to be even more significantly positively associated with highly elevated PSA status, compared to this status among this subset of participants who also were in the lowest half of estimated PhIP intakes, with or without single-variable adjustment for father/brother family history of PC, saturated fat intake, or total energy (Table 4). In particular, this association among men in the highest 15% compared to those in the bottom 50% of estimated PhIP intakes was found to have a maximum-likelihood odds ratio (and corresponding 95% confidence limits) of 32 (3.6, 720), with a corresponding p-value for trend of $p_{\text{trend}}$ = 0.00020. Adjustment for father/brother family history of PC, saturated fat intake, or total energy yielded identical odds-ratio estimates, and only slightly greater estimates of $p_{\text{trend}}$ (Table 4).

A generally similar significant pattern of positive association was also observed between estimated PhIP intake and men with highly elevated PC risk defined as either PSA ≥ 20 ng/mL or a "suspicious" abnormal DRE result leading to medical referral (Table 5). Such suspicious DRE results were obtained among a ~4-fold greater fraction (3 of 12) participants with mildly elevated PSA (≥ 4.0 ng/mL) than among (12 of 370 of) those with PSA < 4.0 ng/mL—a difference that is not statistically significant (p = 0.11 by 2-tail Fisher exact test) perhaps due to study size. Suspicious DRE results were not obtained for any participant with a PSA measure ≥ 20 ng/mL.

Separate trend analyses done to assess for positive association between highly elevated PSA and (either quintiles, or {50, 70, 85}th percentile intervals of) either saturated fat intake, total energy intake, or body mass index, did not yield statistically significant trend-test results with or without adjustment for PhIP intake ($p_{\text{trend}}$ > 0.10 for each of 12 separate tests).

## Discussion

The interim data obtained from the ongoing study described are consistent with the hypothesis that a PSA-related, and a combined PSA- and DRE-related, screening indicator of highly elevated PC risk is significantly

positively associated with estimated dietary exposure to PhIP. Although this conclusion remains tentative in view of the fairly small number of men involved in this prospective study so far, it is supported by the consistency of the pattern of results found, their level of statistical significance, and the significant positive association observed between highly elevated PSA and a positive father/brother history of PC consistent with previous studies that clearly have linked this factor to elevated PC risk (see Introduction).

The results obtained seem to contradict those of the study comparing HA intake (primarily from cooked lamb) and PC risk in New Zeeland men (including 317 PC cases and 480 age-matched controls) studied by Norrish et al. (1999), who found no significant PhIP-related associations involving PC. However, the lower bound on individual-average daily PhIP intake (224 ng/d) that study used in its top exposure quartile that was about 12-fold lower than the lower bound (of about 2680 ng/d) of the top 15% (the highest category) of PhIP exposures estimated for the East San Francisco Bay area African-American men in the present study. If the dose-response for PhIP-induced elevation of PC risk were assumed to be linear no-threshold, linear extrapolation from significantly elevated OR estimates $\geq$ 2.2 or 3.2 obtained in the present study down to PhIP exposure levels used in the Norrish et al. (1999) study would require the latter study to be able to detect elevated OR values as low as about 1.2 or 1.3 in order to have detected a significant PhIP-related association with 95% confidence at 80% statistical power. In contrast, exact evaluation of non-central hypergeometric distributions (Zelterman, 1999) corresponding to the Norrish et al. (1999) study design indicate it could only detect an OR as low as about 1.8 with 95% confidence at 80% power for comparisons involving its lowest vs. highest exposure quartiles. Moreover, the empirical relation between mutagenic and carcinogenic potencies of genotoxic rodent-carcinogen chemicals, such as PhIP, suggests that the low-dose dose-response for cancer induction by such chemicals is likely to be sublinear in general (Bogen, 1993), which implies that the Norrish et al. (1999) design had even less power to detect elevated PC risk due to PhIP exposure levels studied.

Ideally, a prospective study accumulates definitive diagnostic data together with data on exposure- or treatment-related variables of interest. A key limitation to interpretation of data obtained in this study so far is thus that, despite ongoing work to obtain corresponding follow-up diagnostic data, a PC diagnosis is not yet (and for some, may never be) available for all participants who received either positive or highly elevated PC screening results or who received a “suspicious” DRE leading to medical referral. Compared to a case-control design, the prospective design used in this ongoing study has the advantage of being double blind, insofar as PC screening

results analyzed are in each case known neither by the participant nor by study investigators until after each participant provides dietary survey data. This design eliminates potential bias (e.g., in participants' self-reported cooking preferences) associated with knowledge of PC-screening results or PC status, which is important in view of evidence that prior knowledge of cancer-related status can affect dietary recall and so induce significant differential misclassification (Wilkens et al., 1992).

In conclusion, we applied methods for estimating HA concentrations in cooked meats based on individually expressed data on meat-specific intakes, cooking methods and doneness preferences to estimate daily PhIP intakes, and observed these intake estimates to be positively associated with screening indicators of highly elevated PC risk in a prospective clinic-based PC screening study involving nearly 400 African-American men in the San Francisco East Bay area. The observed positive association, which was most significant among men 55 to 70 years of age ($p_{trend} = 0.00020$), remained statistically significant after adjustments for saturated fat intake, total energy intake and self-reported father/brother history of PC. Future work will continue to accrue participants in this study, expand the screening indicators used to predict PC status, and assess whether the observed PhIP-related association pertains as well to incident PC disease.

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**Table 1.** Estimated mean HA intakes by all 21,858 CSFII participants who consumed HAs, by age group.

| Variable[a] | Value(s) | *n* | Mean |
|---|---|---|---|
| Age (y) | 39 to 50 | 115 | 47.2 |
| | 51 to 60 | 216 | 54.6 |
| | 61 to 70 | 61 | 63.6 |
| | All | 392 | 53.9 |
| Weight (kg) | 54.4 to 163 | 392 | 85.7[c] |
| BMI (kg $cm^{-2}$) | <30 | 317 | 25.2 |
| | ≥30 | 75 | 33.9 |
| | 18.4 to 46.0 | 392 | 26.9 |
| PSA (ng/mL) | < 2 | 328 | 0.79 |
| | 2 to <4 | 42 | 2.7 |
| | 4 to <10 | 10 | 5.5 |
| | 10 to <20 | 7 | 14 |
| | ≥20 | 5 | 47 |
| | | PSA<4 | PSA≥4[a] |
| DRE | Normal | 233 | 15 |
| | BPH | 108 | 4 |
| | Suspicious[a] | 12 | 3 |
| Family history[b] | No | 333 | 15 |
| | Yes | 37 | 7 |

[a] BMI = body mass index; DRE = digital rectal exam; Suspicious = abnormal DRE result leading to medical referral; Normal = no urinary, BPH or other symptoms. Each abnormal PSA result (≥4.0 ng/mL) triggered referral for medical follow-up.

[b] Family history = self reported brother(s) and/or father diagnosed with prostate cancer. Association of positive family history with PSA ≥ 4 ng/mL is significantly positive by 2-tail Fisher exact test (p = 0.013).

[c] Weight median (interquartile range) = 83.9 (74.8 to 95.3) kg.

**Table 2**. Summary of meat-specific PhIP intakes for 392 study subjects.

| | Meat type | | | | | | |
|---|---|---|---|---|---|---|---|
| | Chicken | Burger | Beef | Pork | Fish | Bacon | All |
| Mean | 10.3 | 2.54 | 2.39 | 0.34 | 0.36 | 0.02 | 16.7 |
| SD | 12.7 | 3.73 | 4.32 | 0.58 | 1.02 | 0.13 | 16.9 |
| SDM | 0.64 | 0.19 | 0.22 | 0.03 | 0.05 | 0.01 | 0.85 |
| CVM% | 6.24 | 7.41 | 9.12 | 8.22 | 14.6 | 30.1 | 5.11 |
| %All | 61.5 | 15.2 | 14.3 | 2.16 | 2.18 | 0.13 | 100. |

**Table 3**. Association of PhIP intake with elevated PSA.

| Adjustment or restriction[a] | | PSA ≥ 20 ng/mL[b] | | | | | |
|---|---|---|---|---|---|---|---|
| PR | Ave. PhIP intake[a] (ng kg$^{-1}$ d$^{-1}$) | *m* | *n* | OR[c] | 95%LCL[c] | 95%UCL[c] | $p_{trend}$[c] |
| All data | | | | | | | |
| 0-50: | 4.8 | 0 | 196 | 1 | | | |
| >50-70: | 14.6 | 1 | 78 | 7.6 | 0.47 | 200 | |
| >70-85: | 25.5 | 1 | 59 | 10. | 0.62 | 260 | |
| >85-100: | 49.7 | 3 | 59 | 24. | 2.2 | 540 | 0.0024 |
| Adj. for FH | | | | | | | |
| (same as above) | | (*m*, *n*, ORs, LCLs and UCLs unchanged) | | | | | 0.0026 |
| FH – | | | | | | | |
| 0-50: | 4.8 | 0 | 174 | 1 | | | |
| >50-70: | 14.6 | 1 | 69 | 7.6 | 0.47 | 200 | |
| >70-85: | 25.5 | 1 | 53 | 9.8 | 0.61 | 260 | |
| >85-100: | 49.7 | 1 | 52 | 10. | 0.63 | 260 | 0.14 |
| FH + | | | | | | | |
| 0-50: | 4.8 | 0 | 22 | 1 | | | |
| >50-70: | 14.6 | 0 | 9 | – | – | – | |
| >70-85: | 25.5 | 0 | 6 | – | – | – | |
| >85-100: | 49.7 | 2 | 7 | 18. | 1.4 | 540 | 0.0024 |
| Adj. for SatFat | | | | | | | |
| 0-50: | 4.8 | 0 | 196 | 1 | | | |
| >50-70: | 14.6 | 1 | 78 | 3.4 | 0.21 | 89. | |
| >70-85: | 25.5 | 1 | 59 | 4.1 | 0.26 | 110 | |
| >85-100: | 49.7 | 3 | 59 | 24. | 2.2 | 540 | 0.014 |
| Adj. for KCAL | | | | | | | |
| 0-50: | 4.8 | 0 | 196 | 1 | | | |
| >50-70: | 14.6 | 1 | 78 | 14. | 0.85 | 360 | |
| >70-85: | 25.5 | 1 | 59 | 4.3 | 0.27 | 110 | |
| >85-100: | 49.7 | 3 | 59 | 24. | 2.2 | 540 | 0.0030 |

[a] PR = percentile range; FH = family history (self reported brother/s and/or father diagnosed with prostate cancer; SatFat = daily saturated fat intake per kg body weight; KCAL = total energy intake per kg body weight. Trend analyses adjusting for SatFat or KCAL were each done using the adjustment variable dichotomized at its median value.

[b] *m* = number with PSA ≥ 20 ng/mL among *n* total participants included in the analysis.

[c] OR = maximum likelihood odds ratio estimate; CL = confidence limit; LCL = lower CL; UCL = upper CL; $p_{trend}$ = p-value for chi-square test of linear, or (as indicated) adjusted linear, trend.

**Table 4**. Association of PhIP intake with elevated PSA in men ≥ 55 years old.

| Adjustment or restriction[a] / Ave. PhIP intake[a] PR[a] (ng kg$^{-1}$ d$^{-1}$) | PSA ≥ 20 ng/mL[b] *m* | *n* | OR[a] | 95%LCL[a] | 95%UCL[a] | $p_{trend}$[a] |
|---|---|---|---|---|---|---|
| All data | | | | | | |
| 0-50: 4.6 | 0 | 83 | 1 | | | |
| >50-70: 14.4 | 1 | 33 | 7.6 | 0.47 | 200 | |
| >70-85: 24.0 | 0 | 25 | – | – | – | |
| >85-100: 47.7 | 4 | 26 | 32. | 3.2 | 720 | 0.00020 |
| Adj. for FH | | | | | | |
| (same as above) | (*m*, *n*, ORs, LCLs and UCLs approx. unchanged[c]) | | | | | 0.00039 |
| FH – | | | | | | |
| 0-50: 4.6 | 0 | 76 | 1 | | | |
| >50-70: 14.4 | 1 | 27 | 8.5 | 0.52 | 220 | |
| >70-85: 24.0 | 0 | 22 | – | – | – | |
| >85-100: 47.7 | 2 | 22 | 18. | 1.5 | 430 | 0.016 |
| FH + | | | | | | |
| 0-50: 4.6 | 0 | 22 | 1 | | | |
| >50-70: 14.4 | 0 | 9 | – | – | – | |
| >70-85: 24.0 | 0 | 6 | – | – | – | |
| >85-100: 47.7 | 2 | 7 | 18. | 1.4 | 540 | 0.0067 |
| Adj. for SatFat | | | | | | |
| 0-50: 4.6 | 0 | 83 | 1 | | | |
| >50-70: 14.4 | 1 | 33 | 4.1 | 0.25 | 110 | |
| >70-85: 24.0 | 0 | 25 | – | – | – | |
| >85-100: 47.7 | 4 | 26 | 32. | 3.2 | 720 | 0.0024 |
| Adj. for KCAL | | | | | | |
| 0-50: 4.6 | 0 | 83 | 1 | | | |
| >50-70: 14.4 | 1 | 33 | – | – | – | |
| >70-85: 24.0 | 0 | 25 | 4.0 | 0.24 | 110 | |
| >85-100: 47.7 | 4 | 26 | 32. | 3.2 | 720 | 0.0011 |

[a] See Table 3 notes for column-header and acronym definitions.

[b] *m* = number with PSA ≥ 20 ng/mL among *n* total participants included in the analysis.

[c] At mean PhIP-intake level 14.4 ng kg$^{-1}$ d$^{-1}$, OR (95%CL) = 8.5 (0.52, 220).

**Table 5**. Association of PhIP intake with elevated PSA or with suspicious[a] DRE.

| Adjustment or restriction[a] | Ave. PhIP intake[a] (ng kg$^{-1}$ d$^{-1}$) | PSA ≥ 20 ng/mL or suspicious DRE[b] *m* | *n* | OR[a] | 95%LCL[a] | 95%UCL[a] | $p_{trend}$[a] |
|---|---|---|---|---|---|---|---|
| All data | | | | | | | |
| 0-50: | 4.8 | 5 | 187 | 1 | | | |
| >50-70: | 14.6 | 5 | 75 | 2.6 | 0.58 | 11. | |
| >70-85: | 25.5 | 3 | 58 | 2.0 | 0.30 | 11. | |
| >85-100: | 49.7 | 7 | 57 | 5.1. | 1.3 | 21. | 0.0063 |
| Adj. for SatFat | | | | | | | |
| (same as above) | | (*m*, *n*, ORs, LCLs and UCLs unchanged) | | | | | 0.036 |
| Adj. for KCAL | | | | | | | |
| (same as above) | | (*m*, *n*, ORs, LCLs and UCLs unchanged) | | | | | 0.0063 |
| Adj. for FH | | | | | | | |
| (same as above) | | (*m*, *n*, ORs, LCLs and UCLs unchanged) | | | | | 0.0077 |
| FH – | | | | | | | |
| 0-50: | 4.8 | 3 | 168 | 1 | | | |
| >50-70: | 14.6 | 5 | 67 | 4.4 | 0.82 | 29. | |
| >70-85: | 25.5 | 2 | 52 | 2.2 | 0.17 | 20. | |
| >85-100: | 49.7 | 4 | 50 | 4.7 | 0.77 | 34. | 0.066 |
| FH + | | | | | | | |
| 0-50: | 4.8 | 2 | 19 | 1 | | | |
| >50-70: | 14.6 | 0 | 8 | 0.42 | 0.02 | 5.5 | |
| >70-85: | 25.5 | 1 | 6 | 1.7 | 0.02 | 39. | |
| >85-100: | 49.7 | 3 | 7 | 5.8 | 0.50 | 93. | 0.036 |

[a] See Table 3 notes for column-header and acronym definitions. DRE = digital rectal exam; "suspicious" = abnormal DRE result leading to medical referral.

[b] *m* = number with PSA ≥ 20 ng/mL among *n* total participants included in the analysis.

# Figure Legends

**Figure 1.** Cumulative distributions of estimated PhIP intake by meat type for the study population of 392 Oakland, CA-area African-American men. Curves with increasing boldness denote the following meat types (and corresponding % of total estimated intake): burger (15%), other beef (14%), chicken (61%), and total (100%).

**Figure 2.** Ratio of total to basal energy intake (in kcal per kg body weight per day) estimated for the study population of 392 Oakland, CA-area African-American men. The arithmetic mean value (solid line and points) is compared to the expected reference value of 1.6 (dashed lines.

**Figure 3.** PhIP intakes estimated using all meat- and cooking-method-specific doneness information ($PhIP_{in}$), are compared to corresponding intake estimates ($PhIP_{inc}$) each conditioned on meat- and cooking-method-specific PhIP concentrations that were averaged over all participants (i.e., over concentration differences associated with differences in their individually reported doneness preferences). Fitted linear regression (dashed line), with intercept and slope (and corresponding 95%CL) estimates of -0.58 (-1.4, 0.23) and 1.01 (0.974, 1.045), respectively, is not significantly different from an identity relation (solid line).

**Figure 4.** Saturated fat (g/kg-d) intake estimated by FFQ vs. PhIP intake estimated by the LLNL Meat questionnaire for 392 Oakland, CA-area African-American men (open points). Linear regression shown (solid line) indicates a significantly positive association. A single point (closed circle) determined to be an outlier ($p < 10^{-5}$ by F test) was excluded from the regression analysis.

**Figure 5.** Cumulative distributions of PSA measured in 392 Oakland, CA-area African-American men who did (FH +), vs. who who did not (FH –), report a father and/or brothers diagnosed with prostate cancer.

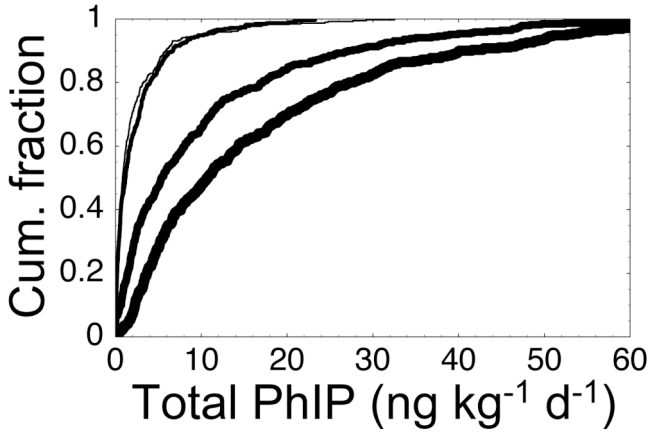
Cum. fraction
1
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
60
Total PhIP (ng kg-1 d-1)

**Figure 1**

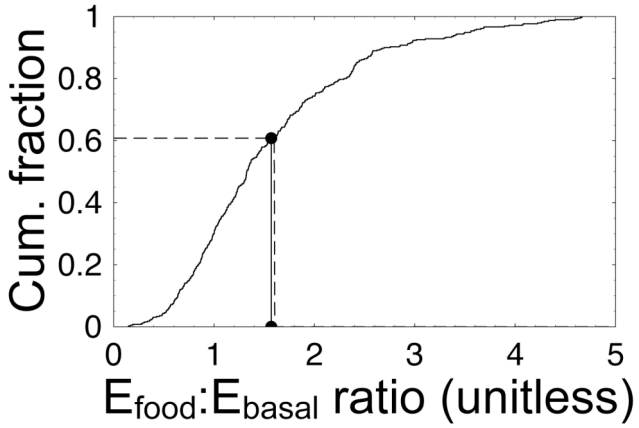
Cum. fraction
1
0.8
0.6
0.4
0.2
0
0
1
2
3
4
5
Efood:Ebasal ratio (unitless)

**Figure 2**

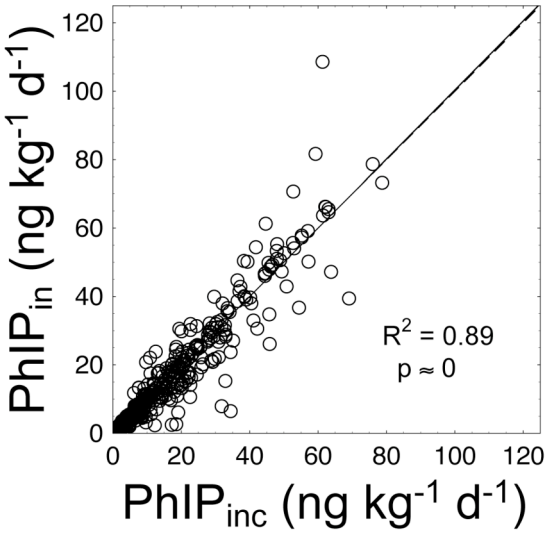


**Figure 3**

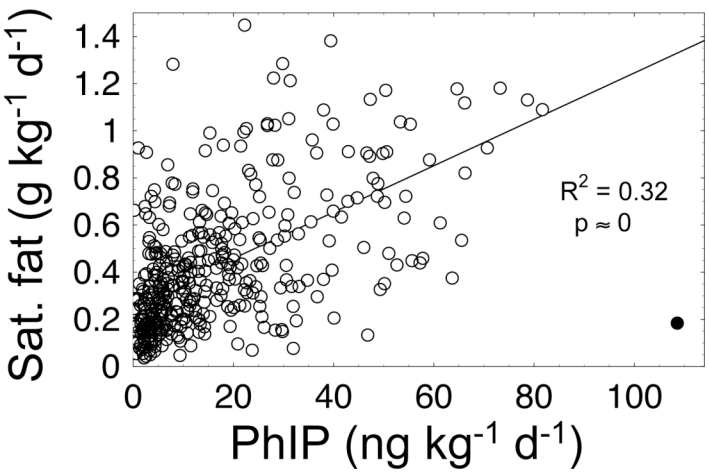
Sat. fat (g kg-1 d-1)
PhIP (ng kg-1 d-1)
R2 = 0.32
p ≈ 0
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0
20
40
60
80
100

**Figure 4**

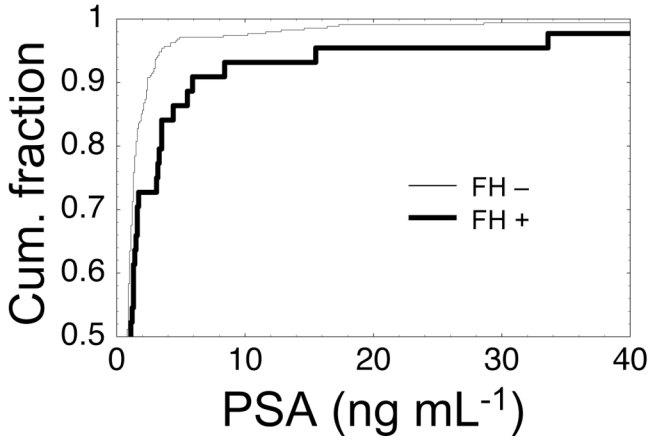


**Figure 5**